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ACUTE KIDNEY INJURY
A STUDY OF FUNCTION MARKERS

Bo Ravn

Karolinska Institutet

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Acute kidney injury, a study of function markers

THESIS FOR DOCTORAL DEGREE (Ph.D.)

Bo Ravn

Principal Supervisor:
Docent Max Bell
Karolinska Institutet
Department of Physiology and Pharmacology
Division of Anesthesia and Intensive Care

Co-supervisor(s):
Docent Johan Mårtensson
Karolinska Institutet
Department of Physiology and Pharmacology
Division of Anesthesia and Intensive Care

Professor Claes-Roland Martling
Karolinska Institutet
Department of Physiology and Pharmacology
Division of Anesthesia and Intensive Care

Opponent:
Professor Peter Pickkers
Radboud University Nijmegen Medical Center
Department of Intensive Care Medicine

Examination Board:
Professor Olav Rooijackers
Karolinska Institutet
Department of CLINTEC
Division of Anesthesia and Intensive Care

Docent Michael Hultström
Akademiska Sjukhuset, Uppsala University Hospital
Department of Medical Cell Biology
Division of Surgical Sciences, Anaesthesiology and Intensive Care

Professor Jan van der Linden
Karolinska Institutet
Department of Molecular Medicine and Surgery (MMK), K1
Division of Thoracic Anesthesia and Intensive Care

ABSTRACT

Acute Kidney Injury (AKI) is defined as a sudden decrease in the kidneys' ability to filtrate waste products and excrete excess water. Detecting AKI primarily relies on measuring the increased concentration of function markers (i.e., creatinine and cystatin C) and even measuring urine output.

This thesis aimed to investigate the performance of kidney function markers in patients during critical illness. To study the variations of the function markers in an ICU population and to examine the performance of the most commonly used estimated glomerular filtration equations. In addition, to investigate the associations between creatinine and cystatin C and long-term mortality and to identify factors predictive of renal dysfunction after ICU discharge.

Both creatinine and cystatin C are within the normally acceptable limits of daily variation which means that changes in function markers between sampling-times during the day are likely to indicate a change in the biomarker levels due to the disease or treatment. Combination of both creatinine and cystatin C enables the best agreement between estimated and measured glomerular filtration rate. Levels of cystatin C after critical illness is strongly associated with 90-day and 1-year mortality in both AKI and non-AKI patients. Creatinine, on the other hand, has little value as a prognostic marker in the majority of patients. The incidence of CKD (eGFR<60) in ICU patients three months after AKI was 25.8% when using creatinine-based eGFR and 63.7% using cystatin C-based eGFR. Creatinine-defined CKD at follow-up was predicted by age, discharge cystatin C, discharge creatinine, and female sex. Cystatin C-defined CKD at follow-up was predicted by age, discharge cystatin C, CRRT in ICU, and diabetes.

LIST OF SCIENTIFIC PAPERS

I. **Intra-day variability of cystatin C, creatinine and estimated GFR in intensive care patients.**
Ravn B, Larsson A, Martensson J, Martling CR, Bell M.
Published: Clin Chim Acta 2016;460:1-4
DOI: 10.1016/j.cca.2016.06.014

II. **Superiority of Serum Cystatin C Over Creatinine in Prediction of Long-Term Prognosis at Discharge From ICU.**
Ravn B, Prowle JR, Martensson J, Martling CR, Bell M.
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III. **Creatinine versus cystatin C based glomerular filtration rate in critically ill patients.**
Ravn B, Rimes-Stigare C, Bell M, Hansson M, Hansson L O, Martling CR, Larsson A, Mårtensson J
DOI: 10.1016/j.jcrc.2019.04.007

IV. **Creatinine- and Cystatin C-Based Incidence of Chronic Kidney Disease and Acute Kidney Disease in AKI Survivors.**
Rimes-Stigare C, Ravn B, Awad A, Torlén K, Martling C-R, Bottai M, Mårtensson J, Bell M.
Published: Critical Care Research and Practice 2018;2018:1-8.
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CONTENTS

LIST OF ABBREVIATIONS

ADQI	Acute Dialysis Quality Initiative
AIC	Akaike Information Criteria
AKD	Acute Kidney Disease
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
APACHE	Acute Physiology and Chronic Health Evaluation II or III
BIC	Bayesian Information Criteria
CI	Confidence Interval
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration.
CRP	C-Reactive Protein
CRRT	Continuous Renal Replacement Therapy
CV	Coefficient of Variation
DR	Swedish cause of death register
ESRD	End Stage Renal Disease
eGFR	Estimated Glomerular Filtration Rate
GFR	Glomerular Filtration Rate
ICD10	International Classification of Diseases, 10^{th} edition
IQR	Interquartile Range
KDIGO	Kidney Disease Improving Global Outcomes group
LM-REV	Lund Malmö creatinine-based eGFR formula
LOS	Length of Stay
MDRD	Modified Diet in Renal Disease eGFR formula
mGFR	Measured Glomerular Filtration Rate
pX	Accuracy to within X% of measured value.
PIN	Personal Identity Number
RIFLE	Risk, Injury, Failure, Loss, and End-stage, AKI classification
RRT	Renal Replacement Therapy
SAPS-3	Simplified Acute Physiology Score-3
SIRS	Systemic Inflammatory Response Syndrome

SAPS	Simplified Acute Physiology Score II or III
SD	Standard Deviation
SNR	Swedish Renal Register

1 INTRODUCTION

Acute kidney injury (AKI) is defined as an acute decline in the kidneys' ability to filter water and waste products. The resulting increase in metabolites, e.g. creatinine and cystatin C is what forms the basis for how kidney function is measured and thereby diagnosed.

Though intense and ongoing research on the topic of kidney biomarkers is being conducted, most hospitals and outpatient clinics and still use creatinine, urea, and urine output as a measure of kidney function. Some clinics have introduced cystatin C as a supplement.

AKI is common during critical illness. It is unclear how these commonly used kidney biomarkers are affected in patients during the time in the intensive care unit.

Considering the most apparent effects of an ICU admission: circadian confusion caused by the ICU milieu, the physiological stress to the body and organs of critical illness, the treatment therapies, and the extensive muscle wasting. No organ goes free not even the kidney.

The aim of this thesis is thus to further investigate the kidney function markers among critical ill patients.

2 BACKGROUND

2.1 DEFINITION OF ACUTE KIDNEY INJURY

Acute kidney injury is a syndrome characterized by a sudden reduction in glomerular filtration rate (GFR). AKI leads to an increase in waste products including the commonly measured creatinine as well as cystatin C and urea.

Though kidney injury has been known for a long period of time, it was not until 2004 that the first successful consensus definition emerged. The definition was known as RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage renal disease) published by the Acute Dialysis Quality Initiative[1]. Simultaneously the term acute renal failure was changed to acute kidney injury in an effort to nuance the spectrum of disease.

The RIFLE criteria were revised by the Acute Kidney Injury Network (AKIN) in 2007 further improving the sensitivity of the diagnostic tool[2].

The latest definition is a unification of both the RIFLE and AKIN criteria presented in 2012 by the Kidney Disease: Improving Global Outcomes (KDIGO)[3].

The definitions are shown in Table 1.

Table 1. Consensus definitions of AKI[4].

Criteria	RIFLE	AKIN	KDIGO
Date of release	2004	2007	2012
Baseline	Not specifically defined. If not available, back-calculate a serum creatinine using an eGFR of 75 ml/min/1.73 m^2 using the MDRD equation	48-h window	Not specifically defined. If not available, use lowest serum creatinine during hospitalization, or calculate SCr using MDRD assuming baseline eGFR 75 ml/min/1.73 m^2 when there is no evidence of CKD
Time interval	Diagnosis and staging: within 1–7 days and sustained more than 24 h	Diagnosis: within 48 h Staging: 1 week	Diagnosis: 50% increase in SCr within 7 days or 0.3 mg/dl (26.5 μmol/l) within 48 h

Criteria		Creatinine	Urine output		Creatinine (urine output criteria same)		Creatinine (urine output criteria same)
Stage	Risk	Increased SCr 1.5–1.9 times baseline or GFR decrease >25%	<0.5 ml/kg/h for 6–12 h	1	Increased SCr 1.5–1.9 times baseline *OR* ⩾0.3 mg/dl (⩾26.5 μmol/l) increase	1	Increased SCr 1.5–1.9 times baseline (7 days) *OR* ⩾0.3 mg/dl (⩾26.5 μmol/l) increase (48 h)
	Injury	2.0–2.9 times baseline or GFR decrease >50%	<0.5 ml/kg/h for ⩾12 h	2	Same as RIFLE minus eGFR criteria	2	same as AKIN
	Failure	3.0 times baseline, GFR decrease >75%, or SCr ⩾4.0 mg/dl (354 μmol/l) with an acute rise of ⩾0.5 mg/dl (44 μmol/l)	<0.3 ml/kg/h for ⩾24 h *OR* Anuria for ⩾12 h	3	Same as RIFLE or on RRT. eGFR criteria removed	3	3.0 times baseline, *OR* Increase in SCr ⩾4.0 mg/dl (354 μmol/l) *OR* Initiation of renal replacement therapy *OR* For <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2
	Loss	Persistent ARF = complete loss of kidney function (need for dialysis) >4 weeks			Notable differences: (1) Addition of 0.3 mg/dl absolute change in SCr to increase diagnostic sensitivity (2) eGFR criteria removed (3) 48-h time window to ensure acuity (also allows for inpatient baseline values) (4) Exclusion of Loss/ESKD categories as diagnostic criteria		Notable differences: (1) Time frame differences for absolute versus relative changes in serum creatinine (2) 0.5 mg/dl increase for those with SCr ⩾4.0 mg/dl (354 μmol/l) no longer required if minimum AKI threshold met (3) Inclusion of eGFR criteria for children
	ESKD	End-stage kidney disease (>3 months)					

Abbreviations: AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; ARF, acute renal failure; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; ESRD, end-stage renal disease; MDRD, Modification of Diet in Renal Disease; KDIGO, Kidney Disease: Improving Global Outcomes; RIFLE, Risk, Injury, Failure, Loss, and End-stage Kidney Disease; SCr, serum creatinine.

Acute Kidney Disease (AKD) proposed in 2017 by ADQI to describe prolonged renal dysfunction after AKI and prior to chronic kidney disease. AKD is considered present if KDIGO-AKI stage 1 or higher is fulfilled during a period of 7 to 90 days after the debut of AKI[5, 6].

Chronic Kidney Disease (CKD) defined by KDIGO guidelines in 2012 as "Abnormality of kidney structure or function, present for more than three months, with implications for health"[3].

End Stage Renal Disease (ESRD) is defined as a severe chronic renal disease with either estimated GFR less than 15ml/min/1.73m^2 or dialysis dependence. Though this definition is a hard endpoint and incidences widely reported in local and national registers it is still disputed[7].

2.2 INCIDENCE AND OUTCOMES

The incidence of AKI varies in the literature depending on the populations studied, and which definitions used.

In the ICU the incidence is increasing[8-10]. In a meta-analysis (data from 2004 to 2012) from 2013, Susantitaphong showed, using the KDIGO definition, that 20% of adults and 33% of children developed AKI during hospitalization[11]. A study[12] with more than 18 million patients with AKI from 2001 to 2011reported an almost fivefold increase in incidence.

Possible explanations are the wider-reaching definitions and increased awareness amongst clinicians and researchers. Additional factors include aging populations, nephrotoxic drugs and treatment regimes, and perhaps even the subtle changes in medical ethics over the years[11, 13, 14].

A Swedish study from 2015 showed that patients with de novo AKI in ICU suffered one-year mortality of 49% while 2% of survivors were dependent on renal replacement therapy[15].

2.3 BIOMARKERS OF RENAL FUNCTION

Renal function is usually expressed in terms of glomerular filtration rate. GFR measures the volume of plasma that is cleared of a substance per unit time (ml/min). Usually GFR is corrected to a standard body surface area of 1.73m^2 [16]. The ability to accurately quantify GFR in critically ill patients remains challenging.

Plasma creatinine is the dominant endogenous marker of GFR used to diagnose and stratify AKI[3]. In addition to being a surrogate measurement of GFR, the plasma creatinine concentration is a function of muscle mass. It is produced from breakdown of creatine-phosphate mainly in skeletal muscles. It is almost freely filtered but also secreted in the tubuli. This being a particular important factor in critically ill patients where continuing loss of muscle mass leads to a parallel decline in plasma creatinine levels and hence a progressive underestimation of true GFR[17].

Measurement of GFR

Measuring GFR using exogenous tracers can be achieved with iohexol, inulin, 99Tm-DTPA, iothalamate, and 51Cr-EDTA. Inulin has traditionally been viewed as the golden standard, but iohexol is on par[18].

In the case of the single injection technique, a series of blood samples is taken at specific time intervals afterward thereby allowing for plotting and calculating both the faster distribution- and the slower elimination-phase[19, 20]. Less exact techniques have been developed where fewer (3 - 5) or even only a single blood sample is needed after injection of, in this case,

iohexol. These techniques are most likely spurred on to minimize the cost and the time-consuming process of a multisample GFR measurement.

The two-compartment method where multiple blood samples obtained both before and after the tracer is completely mixed with the entire distribution volume is considered the most accurate method for measuring GFR using iohexol[21].

Since the use of exogenous markers for measuring GFR is time-consuming and impractical, it is hardly useful in the clinical setting.

Estimation of GFR

Creatinine

Creatinine is a 113 Da amino acid byproduct of the breakdown of creatine phosphate in muscle. Muscle tissue is the primary source of creatine with contribution from dietary intake and liver synthesis. It is not protein-bound and is distributed throughout the total volume of body water. Creatinine excretion is primarily via glomerular filtration where it is freely filtered but also secreted in the tubuli. Approximately 15% is actively secreted by the tubuli, and a small amount is eliminated in the intestines[22].

Creatinine is used worldwide as an endogenous marker for estimating GFR and several eGFR equations exist (Tables 2-4).

The use of creatinine-based eGFR formulas with ICU patients should warrant some concerns. The rapid muscle wasting in critically ill patients reduces the main reservoir of creatine[23]. Even substantial reduction in true GFR can be compensated by increased tubular secretion and compensatory hyperfiltration[24]. Rapid changes in GFR will not show in creatinine concentration because of its large distribution volume.

Endogenous creatinine has been used to estimate GFR since the time of the Second World War, and it is still being used. Probably with good right, in many cases, it delivers a good estimate of the GFR in stable and physically active patients. However, it is probably not to be preferred for use in a population of critically ill patients in the ICU and where early diagnosis of AKI is needed.

Cystatin C

Cystatin C is a 13 kDa enzyme inhibitor involved in protein catabolism and is considered to be produced by all nucleated cells at an almost constant rate.

Elimination from blood is almost exclusively by glomerular filtration[25]. In healthy individuals, cystatin C is freely filtrated and subsequently completely reabsorbed and catabolized by proximal renal tubules and therefore undetectable in urine.

Cystatin C is believed to be a more sensitive marker for GFR than creatinine and has been shown to detect AKI one to two days earlier[26-28]. The plasma levels of cystatin C are affected by Glucocorticoid treatment which increases plasma levels in a dose-dependent manner, and thyroid function where hypothyroid patients have lower levels of cystatin C and hyperthyroid patients have higher levels of cystatin C in plasma.

It has been demonstrated that plasma cystatin C gradual increases during ICU admission in patients with and without AKI[29]. Carlier et al. confirmed that cystatin C based GFR equations systematically underestimated inulin clearance in critically ill patients after a median ICU length of stay of seven days[30].

Iohexol

Iohexol is an iodine-based liquid with low osmolality. In Sweden the most common trade name is Omnipaque. Among other it is used as a contrast agent for radiological examinations. Ironically radiocontrast medium is one of the most common causes of AKI in ICU patients. It is considered essentially free from side effects in low doses [31-33]. Elimination from blood happens by glomerular filtration with no signs of tubular secretion or reabsorption. Because of this it is well suited as a marker for GFR.

When used for measuring GFR the doses of iohexol are 10 – 50 times lower than those used in radiographic examinations, reducing the risk of side effects. Iohexol has been used for GFR measurement in Sweden for more than 15 years.

Table 2. Creatinine based eGFR equations

Creatinine equations
MDRD
Female: $175 * (pCrea/88.4)^{-1.154} * Age^{-0.203} * 0.742$
Male: $175 * (pCrea/88.4)^{-1.154} * Age^{-0.203}$
Revised Lund-Malmö creatinine equation[34] (LM-REV$_{crea}$)
$Exp[X-0.0158 * Age + 0.438 * Ln[Age]]$
Female: pCr < 150: $X = 2.50 + 0.0121 * (150 - pCrea)$
Female: pCr ≥ 150: $X = 2.50 – 0.926 * Ln[pCrea/150]$
Male: pCr < 180: $X = 2.56 + 0.00968 * (180 - pCrea)$
Male: pCr ≥ 180: $X = 2.56 – 0.926 * Ln[pCrea/180]$
CKD-EPI creatinine equation for Caucasians[35] (CKD-EPI$_{crea}$)
Female: pCrea ≤ 62: **CKD-EPI$_{crea}$** $= 144 * (pCrea/62)^{-0.329} * 0.993^{Age}$
Female: pCrea > 62: **CKD-EPI$_{crea}$** $= 144 * (pCrea/62)^{-1.209} * 0.993^{Age}$
Male: pCrea ≤80: **CKD-EPI$_{crea}$** $= 141 * (pCrea/80)^{-0.411} * 0.993^{Age}$
Male: pCrea > 80: **CKD-EPI$_{crea}$** $= 141 * (pCrea/80)^{-1.209} * 0.993^{Age}$

Table 3. Cystatin C based eGFR equations

Cystatin C equations
CAPA[36]
$CAPA_{cysc} = 130 * (pCysC)^{-1.069} * (Age)^{-0.117} - 7$
CKD-EPI cystatin C (CKD-EPI$_{cysc}$)[37]
Male: cystatin C ≤ 0.8: **CKD-EPI$_{cysc}$** $= 133 * (pCysC/0.8)^{-0.499} * 0.996^{Age}$
Male: cystatin C > 0.8: **CKD-EPI$_{cysc}$** $= 133 * (pCysC/0.8)^{-1.328} * 0.996^{Age}$
Female: cystatin C ≤ 0.8: **CKD-EPI$_{cysc}$** $= 133 * (pCysC/0.8)^{-0.499} * 0.996^{Age} * 0.932$
Female: cystatin C > 0.8: **CKD-EPI$_{cysc}$** $= 133 * (pCysC/0.8)^{-1.328} * 0.996^{Age} * 0.932$

Table 4. Creatinine and Cystatin C based eGFR equations

Combination (Creatinine and Cystatin C) equations[37]
Female:
pCrea ≤ 62 & pCysC ≤ 0.8:
$CKD\text{-}EPI_{crea+cysc} = 130 * (pCrea/62)^{-0.248} * (pCysC/0.8)^{-0.375} * 0.995^{Age}$
pCrea ≤ 62 & pCysC > 0.8:
$CKD\text{-}EPI_{crea+cysc} = 130 * (pCrea/62)^{-0.248} * (pCysC/0.8)^{-0.711} * 0.995^{Age}$
pCrea > 62 & pCysC ≤ 0.8:
$CKD\text{-}EPI_{crea+cysc} = 130 * (pCrea/62)^{-0.601} * (pCysC/0.8)^{-0.375} * 0.995^{Age}$
pCrea > 62 & pCysC > 0.8:
$CKD\text{-}EPI_{crea+cysc} = 130 * (pCrea/62)^{-0.601} * (pCysC/0.8)^{-0.711} * 0.995^{Age}$
Male:
pCrea ≤ 80 & pCysC < 0.8:
$CKD\text{-}EPI_{crea+cysc} = 135 * (pCrea/80)^{-0.207} * (pCysC/0.8)^{-0.375} * 0.995^{Age}$
pCrea ≤ 80 & pCysC > 0.8:
$CKD\text{-}EPI_{crea+cysc} = 135 * (pCrea/80)^{-0.207} * (pCysC/0.8)^{-0.711} * 0.995^{Age}$
pCrea > 80 & pCysC < 0.8:
$CKD\text{-}EPI_{crea+cysc} = 135 * (pCrea/80)^{-0.601} * (pCysC/0.8)^{-0.375} * 0.995^{Age}$
pCrea > 80 & pCysC > 0.8:
$CKD\text{-}EPI_{crea+cysc} = 135 * (pCrea/80)^{-0.601} * (pCysC/0.8)^{-0.711} * 0.995^{Age}$

2.4 CIRCADIAN RYTHMS

Circadian rhythms (from Latin: circa "about" and dies "day") refer to the near 24-hour physiological processes in humans. The functions of the body synchronize with each other and the external environment. This circadian pacemaker is located in the suprachiasmatic nuclei in the hypothalamus.

The influence of alternating light and darkness and other cues in everyday healthy subjects helps to keep a fixed temporal relationship with the environment. If this relationship is affected the "clock" tends to drift with an intrinsic period of 24.18 hours[38]. Evidence supports that the pacemaker property remains stable with age.

A study of circadian variability in healthy subjects showed that neither creatinine nor cystatin C differed significantly between night- and day-sleep conditions[39]. Concluding that sampling of these two markers does not have to be restricted to specific times of the day.

The ICU milieu is quite harsh on the body and mind. Besides the critical illness affecting organ(s) patients are often medically sedated to various degrees. Ambient environment is often noisy and light. These factors alone can cause desynchronization of the circadian pacemaker[40]. Evidence has shown that patients with higher APACHE III scores show greater circadian phase displacement[41].

3 AIMS OF THE THESIS

The general aim was to investigate the performance of kidney function markers in patients during critical illness.

Study I: To study the intraday variation of kidney function markers in ICU patients.

Study II: To study the associations between creatinine & cystatin C and long-term mortality.

Study III: To study the performance of commonly used estimated GFR equations in an ICU context.

Study IV: To study the incidence of CKD both creatinine and cystatin C based. To investigate factors predictive of renal dysfunction at three months according to creatinine and cystatin C.

4 SUBJECTS AND METHODS

4.1 ETHICAL CONSIDERATIONS

The Regional Ethics Committee of Stockholm approved studies I – IV. The studies were performed in compliance with the Helsinki Declaration of 1964 and its amendments. For study I and III written informed consent was obtained from patients or next of kin before enrollment.

4.2 REGISTERS AND DATABASES

The Swedish National Registration Number

A unique Personal Identification Number (PIN)[42] is issued to all Swedish citizens at birth or upon immigration. It is used for interactions with government and private administrative agencies including health care. It allows for linkage with other registers e.g. The Cause of Death Register.

The Cause of Death Register

The Cause of Death Register was established in 1952 and is managed by the Swedish Board of Health and Welfare. It contains the time and cause of death for all residents who have been issued with a national identification number. The register is considered very reliable. Ref 2010 socialstyrelsen dödorsaksstatistik[43].

This database was used in study II and IV

Centricity Clinisoft, The patient Database Management System in the ICU

Centricity Clinisoft is a Patient Database Management System developed by GE Healthcare. It acquires and stores physiological data from patients as well as admission/discharge times, laboratory results, ventilator settings, diagnoses, and to a limited degree procedural codes and treatments.

Clinisoft was incrementally introduced ICUs at Karolinska University Hospital starting in 2005 and less than two years later implemented in all ICU departments.

This database was used in study II and IV

EXCRETe

The EXCRETe (EXtracorporeal Clearance & REsidual renal function during rrT) database is Excel based and includes patients with eGFR (MDRD estimate) > 60mL/min/1.73m^2 on admission to ICU (CIVA) and with an expected length of stay above 24h. Exclusion criteria are patients with RRT treatment prior to ICU admission. The first EXCRETe patient was included November 2008.

Data consists of daily scoring of RIFLE and AKIN (both creatinine and urine output criteria); SIRS; baseline characteristics APACHE II-score (Acute Physiology And Chronic Health Evaluation), ICU diagnoses; urinary output; blood pressure; biomarker concentrations; body weight; drug treatments.

This database was used in study IV

Take Care

Take Care (CompuGroup Medical, Koblenz, Germany) is a hospital electronic health records system which is used by several hospitals and outpatient clinics in Sweden. It contains medical journals, laboratory results, consultant statements, and results from diagnostic tests.

4.3 SUMMARY OF STUDIES I – IV

	Study I	Study II	Study III	Study IV
Data source	General ICU and Neurosurgical ICU, TC, Clinisoft	Clinisoft, DR, SNR	General ICU and Neurosurgical ICU, TC, Clinisoft	Clinisoft, Excrete, DR, SNR
Design	Single center Prospective cohort	Single center Retrospective observational cohort	Single center Prospective cohort	Single center Prospective cohort
Study period	2013-2014	2006 – 2013	2013-2014	2008-2011
Number of subjects	28	3077	30	274
Outcome	eGFR Creatinine Cystatin C	Creatinine, Cystatin C, eGFR Death	mGFR eGFR	CKD at three months Death
Analyses	CV	Multivariable survival model, HR,	eGFR, Iohexol measurements of GFR	Cox regression Kaplan Meier Logistic regression

4.4 STUDY I

Design and study population

The cohort consisted of 28 patients. The period of inclusion was between January 2013 and September 2014.

Inclusion criteria were clinically stable patients treated at two intensive care units at Karolinska University Hospital. The patients had been treated at the ICU for a period longer than three days, and they were expected to stay at the ICU for additional 24h after inclusion, were not in a septic state, and did not require active fluid resuscitation.

Exclusion criteria were patients under 18 years of age, unwillingness to participate (the patient him- or herself or next of kin), planned surgery during the time of the investigation, pregnancy, patients treated with renal replacement therapy, and patients who had been exposed to contrast media during the time of treatment in the ICU.

Statistical analysis

Continuous data are reported as mean or median. The coefficient of variation was defined as the standard deviation (SD) divided with the mean of all results for each patient.

For estimating GFR for creatinine the CKD-EPI equation was used and for estimating GFR for cystatin C the CAPA equation was used. Additionally, a combination of creatinine and cystatin C formula was used.

4.5 STUDY II

Design and study population

A total of 3077 patients were included into the study during the period November 2006 – December 2013. All ICU admissions greater than 24 hours were eligible for inclusion. Using the Personal Identification Number patient data was linked with diagnosis data, pre- and post ICU laboratory data (TC), the Cause of Death Register, and with the Swedish Renal Registry. Thus allowing for evaluation of pre-ICU Charlson Comorbidity Index (Quan modification), exclusion of patients with ESRD prior to or within 14 days after ICU discharge, and patients who died in the ICU or within three days of discharge. (Figure 1)

AKI was defined according to the Kidney Disease: Improving Global Outcomes criteria i.e. a 1.5 fold increase from baseline serum creatinine at ICU admission or an absolute creatinine increase of 26.5mcr mol/L within a 48 period. (Table 1)

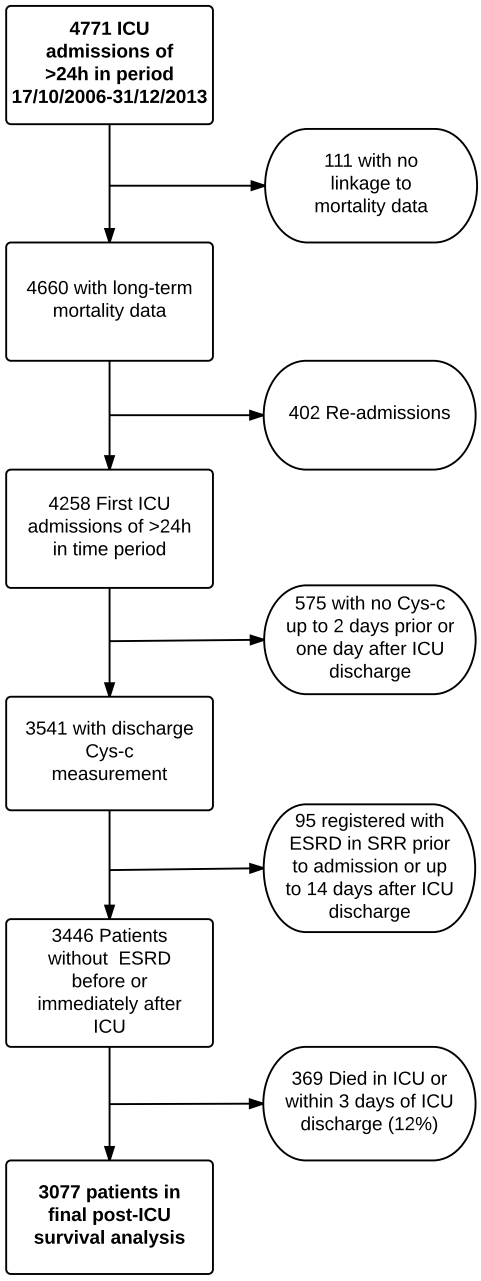

Figure 1. Study II – Patient selection flowchart.

Statistical analysis

Continuous variables were presented as median with interquartile range. Wilcoxon rank-sum test, Wilcoxon signed-rank test, and Fishers exact test were used for univariable comparisons. Fisher exact test was used for binary and continuous data. Kaplan-Meier plots of unadjusted survival data which were stratified by quartiles of creatinine and cystatin C.

4.6 STUDY III

Design and study population

The cohort consisted of 30 patients. The period of inclusion was between January 2013 and September 2014.

Inclusion criteria were clinically stable patients treated at two intensive care units at Karolinska University Hospital. The patients had been treated at the ICU for a period longer than three days, and they were expected to stay at the ICU for additional 24h after inclusion, were not in a septic state, and did not require active fluid resuscitation.

Exclusion criteria were patients under 18 years of age, unwillingness to participate (the patient him- or herself or next of kin), planned surgery during the time of the investigation, pregnancy, patients treated with renal replacement therapy, and patients who had been exposed to contrast media during the time of treatment in the ICU.

Statistical analysis

Five single markers estimating GFR equations were used. Three creatinine-based equations: $MDRD_{CREA}$, $LM\text{-}REV_{CREA}$, and $CKD\text{-}EPI_{CREA}$. Two cystatin C-based equations: $CAPA_{CYSC}$ and $CKD\text{-}EPI_{CYSC}$.

Three combined equations were used: $CKD\text{-}EPI_{CREA+CYSC}$, Mean of CAPA and LM-REV, and Mean of the two single-marker CDK-EPI equations.

Continuous variables were presented as median with interquartile range and categorical variables as n (%). Bias was estimated with the median differences between eGFR and measured GFR. Precision was estimated as the interquartile range of the difference eGFR and mGFR. 95%-CI was estimated using Efron's non-parametric bias corrected and accelerated (BCa) method.

Accuracy was assessed as the absolute difference between eGFR and mGFR as well as the proportion of eGFR within 10%, 20% and 30% of mGFR presented as P10, P20, and P30 respectively. CIs were assessed by using the binomial proportion Wilson score interval.

Bland-Altman plots representing the agreement between eGFR and mGFR (presented as supplemental material to the article).

4.7 STUDY IV

Design and study population

1869 patients were admitted to the ICU during the study time. After exclusion, death, lost to follow-up, and patients not feeling well enough to attend the result was a prospective cohort of 274 patients. Study-inclusion period was between 2008 and 2010

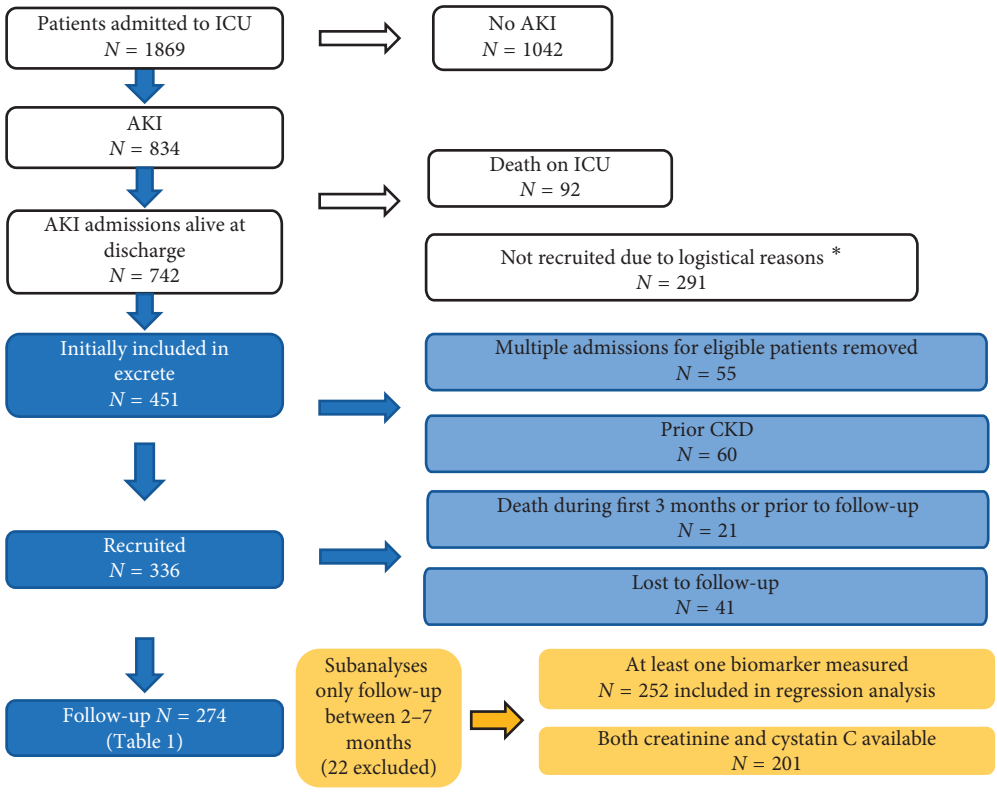

Figure 2. Flow chart showing selection and exclusion of patients in the follow-up cohort. White boxes: Entire ICU cohort, information derived from cross-matching with the ICU database and death register. Blue boxes: Data from the initial study database. Yellow boxes: Details of groups included in subanalyses. * Patients discharged when research staff were not working or who were transferred to other hospitals were not recruited[44].

Inclusion criteria were patients with AKI (defined according to RIFLE[1] which was the classification system used at the time of the study) during admission to and alive at discharge from the general ICU at Karolinska University Hospital Solna.

Exclusion criteria were age younger than 18 and older than 100 years. Death within three months after discharge from ICU. Presence of CKD or ESRD prior to ICU admission. Loss of recruitment due to lack of research staff availability during weekends, public holidays, and the period from February to April 2010.

At three months follow-up patients were referred to either nephrology or ICU outpatient clinics where creatinine and cystatin C were measured. Patients who did not attend follow-up were reviewed and values from visits to primary care were collected.

Statistical analysis

Continuous variables were presented as median with interquartile range. Categorical data expressed as counts with percentage. Distributions of continuous variables were compared using Mann-Whitney's test while Fisher's test was used for comparing means of binary variables. A two-sided p-value < 0.05 was considered statistically significant.

For estimating creatinine-based GFR the MDRD, LM-REV, and CDK-EPI while cystatin C-based GFR was calculated with the CDK-EPI (cystatin C). The composite CKD-EPI (creatinine, cystatin C) was also used.

The patients were classified as having CKD at three months according to KDOQI stages of chronic kidney disease. Urine-based eGFR was not used[45]. Patients without prior CKD were classified as having AKD if their follow-up creatinine was >1.5x their baseline creatinine.

Modeling was done in the following way. Death was considered a censoring event. A Cox regression model weighted for the inverse probability of dying after discharge and before three months. It was adjusted for covariates found to be independently associated with death before follow-up. Variables included in the final model were: age, sex, and maximum RIFLE stage. This model was used in all regression analyses.

Logistic regression was used to identify covariates which affected the risk of the binary outcome of CKD ($<60 ml/min/1.73m^2$) according to creatinine and cystatin C. Kaplan-Meier was used to calculate survival probabilities, differences between groups were tested using the log-rank test. The models were assessed using Somers' d, Bayesian-, and Akaike information criterion (BIC, AIC).

5 RESULTS AND DISCUSSION

5.1 STUDY I

Results

The coefficient of variation was marginally larger for creatinine than for cystatin C (3.70% respective 3.66%). CV for the corresponding creatinine and cystatin C eGFR were 2.00% respective 4.60%. See table 6.

Comparing the eGFR, cystatin C consequently showed values nearly half of that of creatinine.

Table 5.

	Median concentration	Median eGFR (mL/min/1.73m^2)	Median CV (conc)	Median CV eGFR
Creatinine	50 umol/L	102.8	3.70%	2.00%
Cystatin C	1.44 mg/L	47.2	3.66%	4.60%

Discussion

This study showed that the median coefficient of variation of creatinine, cystatin C, and of eGFR (both creatinine and cystatin C based) was below 5%. This means that 95% of the results will vary with less than 10% between sampling times.

Differences larger than 10% between sampling times are therefore more likely to be an indication of changes in biomarker levels due to disease and or treatment.

5.2 STUDY II

Results

At 90-days after ICU discharge 318 (10.3%) had died and at one year this number had risen to 536 (17.4%). (Table 7). Nonsurvivors who more often developed AKI were older, had higher Simplified Acute Physiology Score-3 (SAPS-3) and had more comorbidities. From ICU admission to discharge creatinine fell and cystatin C increased in value.

Cystatin C and creatinine differed distinctly in their association with mortality at 90-day and 1-year at ICU discharge Table 8. Comparing the unadjusted quartile 1 for creatinine (13.6%) and cystatin C (5.6%) and marks a low-risk population for cystatin C.

When fitting creatinine and cystatin C to penalized splines in a multivariable survival model (sex, age-stratification, comorbidity index) it shows that cystatin C was near linearly related to an increased hazard ratio for death. Creatinine showed a less linear relationship with a more flat curve (Figure 4).

Analyzing both creatinine and cystatin C together in a new multivariable survival model (sex, age-stratification, comorbidity index) showed a strong association between increasing cystatin C and hazard ratio for death. The opposite for creatinine which now showed a lower hazard ratio with increasing discharge concentration. (Figure 5).

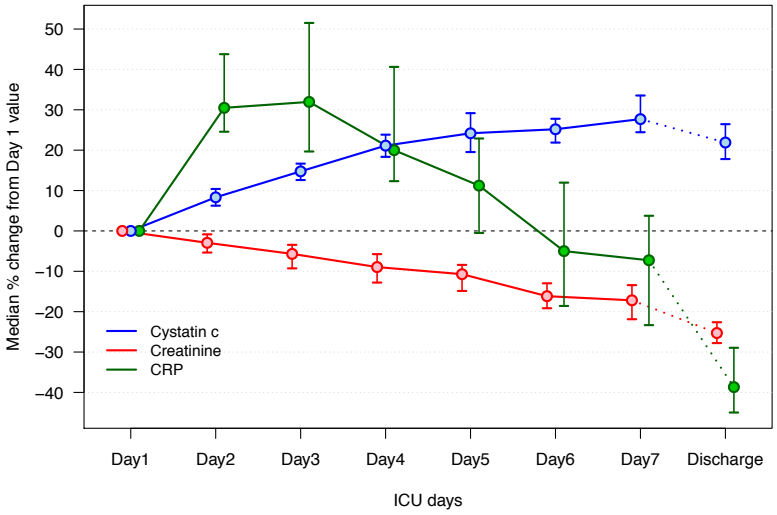

Figure 3. Time-course of creatinine, cystatin C, and C-Reactive Protein in 516 patients with ICU admissions greater than 7 days. (Median % change from d1 value and binomial estimate of the 95% CI of the Median).

Cystatin C based eGFR identified, at discharge, 1,362 patients (44%) with a GFR < 60ml/min/1.73m^2 where creatinine-based eGFR identified 794 (26%).

In a subset of 516 patients with a length of stay of 7 days or greater creatinine, cystatin C, and C-reactive protein (CRP) were compared (Figure 2). Creatinine had a clear downward slope while cystatin C progressively rose. CRP showed a bi-phasic profile that did not correlate with the two endogenous renal filtration markers.

Table 6 Demographics and Renal Filtration Markers in Survivors and Non-survivors

Characteristics	All Patients, *n* (%) or Median (IQR)	Survived 1 yr, *n* (%) or Median (IQR)	Died by 1 yr, *n* (%) or Median (IQR)
Number	3,077 (100)	2,542 (82.6)	535 (17.4)
Age	59 (41–70)	56 (38–68)	69 (60–76)
Male sex	1,899 (61.7)	1,559 (61.3)	340 (63.4)
ICU length of stay (hr)	58 (34–120)	58 (33–119)	62 (37–125)
Simplified Acute Physiology Score-3[a]	37 (26–50)	35 (24–48)	47 (36–57)
Medical	1,348 (43.8)	1,039 (40.9)	309 (57.8)
Surgical	959 (31.2)	776 (30.5)	183 (34.2)
Trauma	770 (25.0)	727 (28.6)	43 (8.0)
Infection (primary diagnosis)	704 (22.8)	520 (20.5)	184 (34.4)
Closest to ICU admission creatinine	0.98 (0.74–1.41)	0.96 (0.74–1.38)	1.14 (0.80–1.73)
Closest to ICU admission cystatin C[a]	1.01 (0.75–1.53)	0.94 (0.72–1.38)	1.36 (1.02–1.93)
Peak creatinine	1.06 (0.79–1.66)	1.03 (0.77–1.56)	1.30 (0.87–2.15)
Peak cystatin C	1.20 (0.83–1.93)	1.11 (0.80–1.76)	1.69 (1.19–2.52)
Discharge creatinine	0.83 (0.63–1.20)	0.80 (0.62–1.13)	1.02 (0.69–1.57)
Discharge cystatin C	1.10 (0.80–1.63)	1.01 (0.77–1.48)	1.57 (1.12–2.16)
Discharge eGFR (creatinine)	92 (58–112)	95 (65–115)	70 (41–96)
Discharge eGFR (cystatin C)	68 (37–105)	76 (44–109)	40 (26–64)
Renal replacement therapy in ICU	238 (7.7)	177 (7.0)	61 (11.4)
Comorbidities			
Myocardial infarction	240 (7.82)	163 (6.41)	77 (14.39)
Congestive heart failure	289 (9.42)	189 (7.44)	100 (18.69)
Peripheral vascular disease	330 (10.75)	256 (10.07)	74 (13.83)
Stroke	237 (7.72)	169 (6.65)	68 (12.71)
Dementia	32 (1.04)	11 (0.43)	21 (3.93)
Pulmonary disease	405 (13.2)	308 (12.12)	97 (18.13)
Rheumatic disease	102 (3.32)	76 (2.99)	26 (4.86)
Peptic ulcer disease	94 (3.06)	60 (2.36)	34 (6.36)
DM without complication	310 (10.1)	243 (9.56)	67 (12.52)
DM with complication	112 (3.65)	76 (2.99)	36 (6.73)
Hemiplegia or paraplegia	87 (2.83)	69 (2.71)	18 (3.36)
Renal disease	142 (4.63)	102 (4.01)	40 (7.48)
Liver disease mild	108 (3.52)	94 (3.7)	14 (2.62)
Liver disease moderate-severe	39 (1.27)	20 (0.79)	19 (3.55)
Nonmetastatic cancer	478 (15.58)	339 (13.34)	139 (25.98)
Metastatic cancer	317 (10.33)	188 (7.4)	129 (24.11)
HIV	11 (0.36)	9 (0.35)	2 (0.37)
Charlson Comorbidity Index (Quan)			
0	1,591 (51.71)	1,472 (57.91)	119 (22.24)
1–2	781 (25.38)	623 (24.51)	158 (29.53)
3–4	314 (10.21)	219 (8.62)	95 (17.76)
5–6	293 (9.52)	177 (6.96)	116 (21.68)
> 6	98 (3.19)	51 (2.01)	47 (8.79)
AKI			
No AKI	2,157 (70.10)	1,848 (72.70)	309 (57.76)
AKI-1	408 (13.26)	319 (12.55)	89 (16.64)
AKI-2	172 (5.59)	116 (4.56)	56 (10.47)
AKI-3	340 (11.05)	259 (10.19)	81 (15.14)

AKI = acute kidney injury, DM = diabetes mellitus, eGFR = estimated glomerular filtration rate, IQR = interquartile range.
[a]Values available in 3,013 patients for Simplified Acute Physiology Score-3 and 3,070 for closest to admission cystatin C.

Table 7 Crude mortality and Adjusted Hazard Ratios for Death over 90-days and 1-Year Follow-up After Critical Illness Associated with plasma Cystatin C and Creatinine Measurements Near ICU Discharge

	Unadjusted Post-ICU Survival by Quartile of Cystatin C or Creatinine				Adjusted Hazard Ratio 75th Relative to 25th Centile
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Cystatin C					
Values (mg/L)	0.20–0.80	0.80–1.10	1.10–1.63	1.63–8.48	1.63 vs 0.80
90-d mortality	2.6%	6.2%	11.8%	21.0%	2.23 (1.63–3.02)
365-d mortality	5.6%	11.0%	21.2%	32.0%	1.78 (1.46–2.18)
Creatinine					
Values (mg/dL)	0.1–0.63	0.63–0.83	0.83–1.20	1.20–11.55	1.20 vs 0.63
90-d mortality	8.4%	6.3%	9.9%	17.0%	1.09 (0.89–1.33)
365-d mortality	13.6%	12.4%	17.3%	26.7%	1.03 (0.87–1.21)

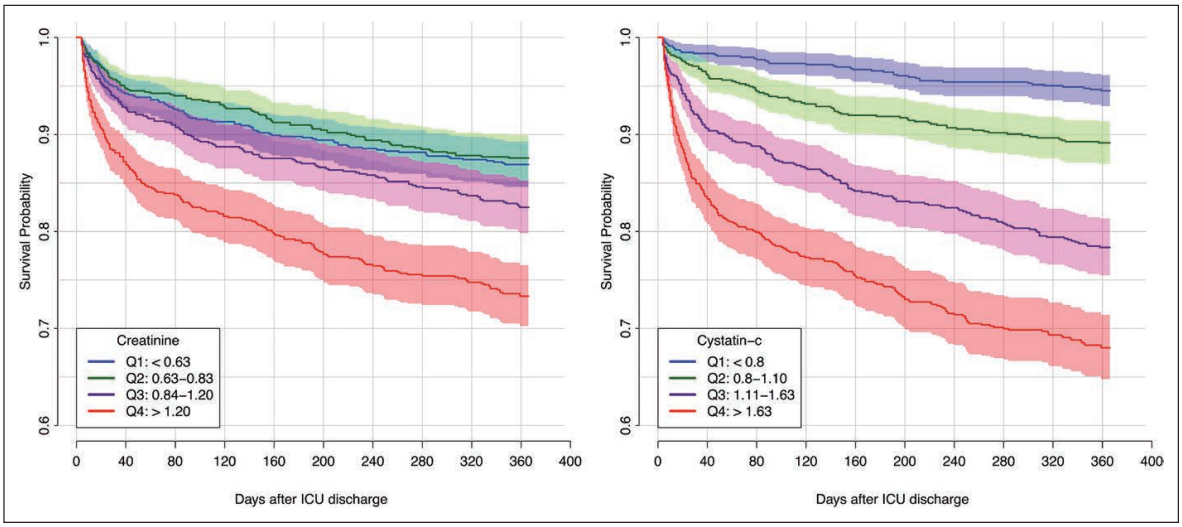

Figure 4. Crude mortality in the year after ICU discharge stratified by quartiles of creatinine or cystatin C at ICU discharge.

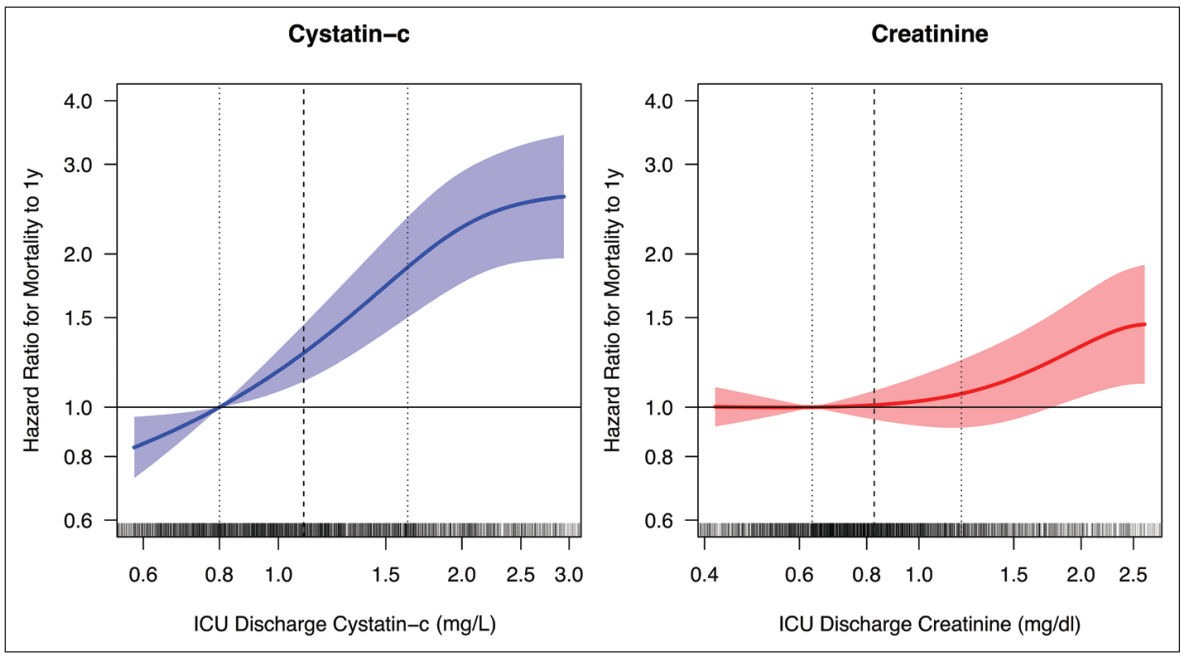

Figure 5. Age- and sex-adjusted hazard ratios for survival in the year after ICU discharge.

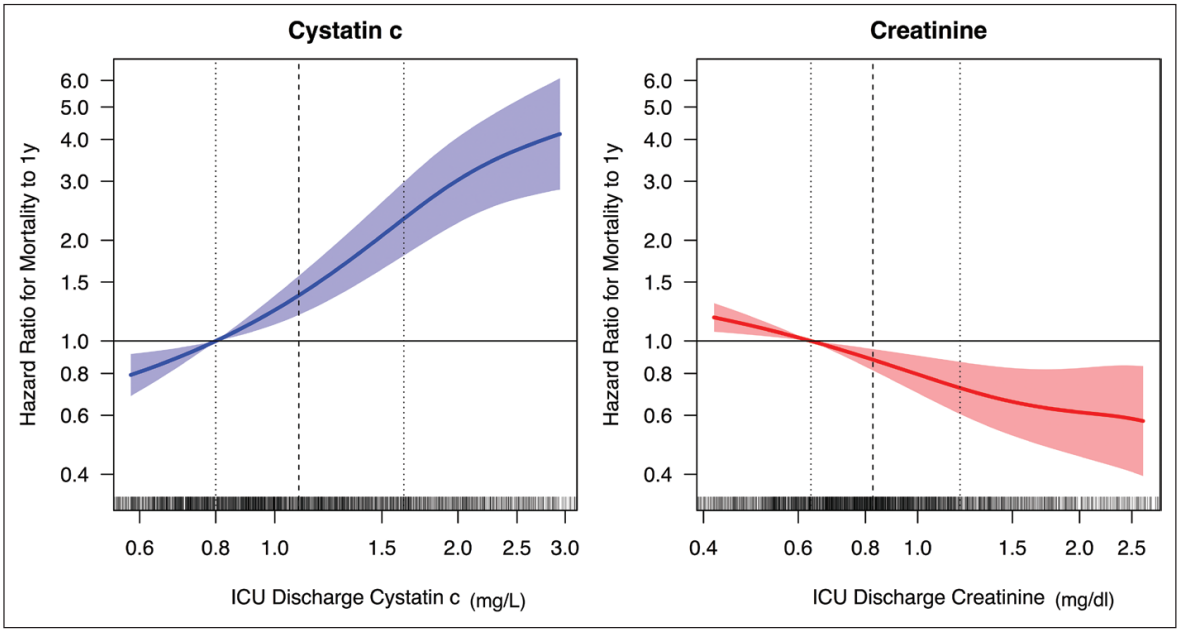

Figure 6. Including cystatin C and creatinine together in a single Cox proportional hazard survival model, age- and sex-adjusted hazard ratios for survival in the year after ICU discharge fitted with penalized spline regression for ICU discharge creatinine adjusted for cystatin C and cystatin C adjusted for creatinine.

Discussion

Creatinine and cystatin C diverge during ICU admission as seen in the subgroup of 516 patients in figure 2. At the time of ICU discharge, the median difference between eGFR (cystatin C) and eGFR (creatinine) was 24ml/min/1.73m^2. So clearly one, or both, fail to reflect the GFR.

Assuming that both kidney function markers are similarly affected by renal function then the difference must be explained by a difference in the rate of generation of the markers during critical illness.

Age- and sex-adjusted hazard ratios for survival in the year after ICU discharge is shown in figure 4. The linearity presented by cystatin C shows a clear increased risk of death not produced by creatinine.

At shorter ICU admissions creatinine and cystatin C eGFR were similar.

A subgroup of 743 patients without AKI in the ICU survived up to one year after hospital discharge. Creatinine was measured between 30 to 365 days. Comparing these patients out-of-hospital eGFR (creatinine) with the discharge eGFR (both creatinine and cystatin C) showed that cystatin C based eGFR at ICU discharge was closer to the creatinine-based

eGFR measured after discharge. This was significant for the GFR interval 30 to 60ml/min/1.73m^2.

In other words, at ICU discharge the cystatin C predicts creatinine eGFR better than creatinine does at ICU discharge for this subgroup.

The reduction in creatinine generation with muscle wasting may explain why lower discharge creatinine is not associated with better prognosis.

When combining both markers in a single Cox proportional hazard survival model (figure 5) cystatin C remained strongly associated with mortality and increasing creatinine was consistently associated with lower mortality. Potentially reflecting lower mortality in patients with less muscle wasting.

5.3 STUDY III

Results

The performance of both single and combination GFR endogenous biomarker estimates is shown in table 9. The creatinine-based eGFR equations (MDRD, LM-REV, and CKD-EPIcr) overestimated mGFR while cystatin C-based eGFR equations (CAPA and CKD-EPIcysc) underestimated mGFR.

Single marker estimates: LM-REV demonstrated the lowest bias (8.0ml/min/1.73m^2) and best p30 accuracy (66.7%). MDRD performed the worst with the greatest bias, the lowest precision, and the lowest accuracy.

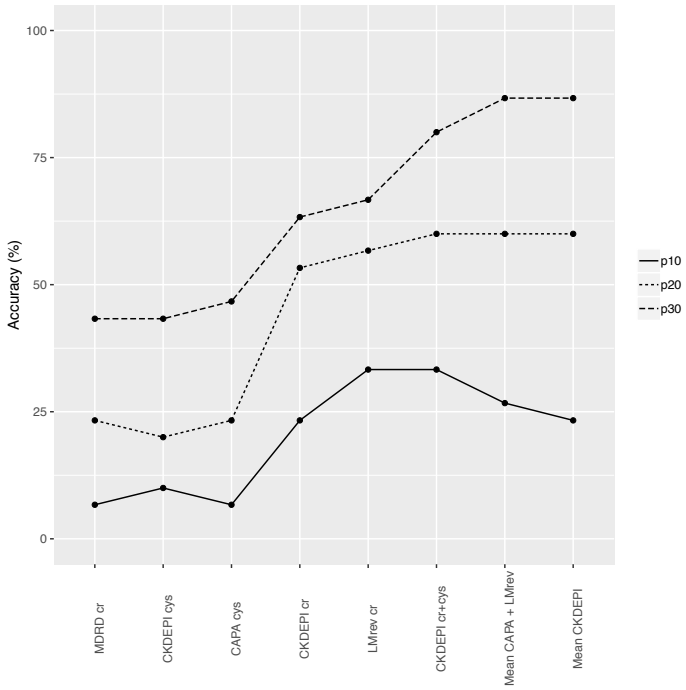

Figure 7. Accuracy of each of the eGFR equations expressed as percentage within 10%, 20% or 30% (p10, p20, p30 respectively) of measured GFR

The combined eGFR equations underestimated measured GFR (median of 7.4 to 11.5ml/min/1.73m^2). When compared with the single marker estimates the combined eGFR equations showed consistently higher p30, p20 accuracy (figure 6) although they were inferior to the cystatin C single marker in their precision.

Table 8. Performance of single marker and combined marker equations calculated from the differences between estimated and measured GFR (estimated minus measured GFR, i.e. positive values if the estimate is higher than the measured).

	Single marker equations					Combined marker equations		
Performance	**$MDRD_{CREA}$**	**LM-REV_{CREA}**	**$CAPA_{CYSC}$**	**CKD-EPI_{CREA}**	**CKD-EPI_{CYSC}**	**CKD-$EPI_{CREA+CYSC}$**	**$MEAN_{CAPA+LM-REV}$**	**$MEAN_{CKD-EPI}$**
Bias								
Median difference[a], ml/min/1.73 m^2 (95% CI)	35.77 (18.1 to 47.1)	8.0 (-4.17 to 16.2)	- 26 (-30.4 to -19.7)	14 (2.16 to 24.1)	-25 (-32.3 to -20.3)	-10 (-17.1 to -0.648)	-11.5 (-19.6 to -0.0541)	-7.38 (-14.7 to 4.42)
Median percentage difference (95% CI)	49.5 (24.1 to 78.9)	9.3 (-4.8 to 24.4)	-32.2 (-41.4 to -25.1)	18.7 (1.2 to 33.5)	-33.1 (-44.7 to -24.6)	-12.1 (-19.2 to -1.2)	-13.8 (-19.4 to -0.04)	-8.7 (-15.9 to 4.98)
Precision								
IQR of differences[b], ml/min/1.73 m^2 (95% CI)	45.5 (24.95 to 80.41)	31.6 (19.9 to 44.4)	18.3 (11.5 to 33.3)	35.9 (24.0 to 50.1)	18.0 (8.6 to 29.3)	20.8 (15.8 to 32.5)	22.9 (15.7 to 31.7)	25.0 (18.8 to 35.6)
Accuracy								
Median absolute percentage difference (95% CI)	49.5 (24.6 to 77.7)	17.1 (9.8 to 27.3)	32.2 (24.6 to 40.6)	19.6 (12.4 to 33.5)	33.1 (23.7 to 42.2)	14.5 (10.5 to 24.6)	17.5 (12.8 to 21.4)	16.1 (10.6 to 21.9)
p30, %[c] (95% CI)	43.3 (27.4 to 60.8)	66.7 (48.8 to 80.8)	46.7 (30.2 to 63.9)	63.3 (45.5 to 78.1)	43.3 (27.4 to 60.8)	80.0 (62.7 to 90.5)	86.7 (70.3 to 94.7)	86.7 (70.3 to 94.7)
p20, %[c] (95% CI)	23.3 (12.0 to 40.9)	56.7 (39.2 to 72.6)	23.3 (11.8 to 40.9)	53.3 (36.1 to 69.8)	20.0 (9.5 to 37.3)	60.0 (42.3 to 75.4)	60.0 (42.3 to 75.4)	60.0 (42.3 to 75.4)

CI, confidence interval; IQR, interquartile range
Measured GFR denotes the iohexol-based measurement of GFR.
[a]Difference in estimated minus measured GFR.
[b]Interquartile rage (IQR) defined as the range between Q1 and Q3.
[c]p30 and p20 refer to the percentage of GFR estimates within 30% and 20% of measured GFR, respectively

Discussion

Iohexol measurements were performed after a median length in the ICU of 16 days (10 - 21). Which is a long time considering how fast immobilization or partial immobilization affects muscle volume. The creatinine-based equations overestimated and the cystatin C-based equations underestimated GFR. The "native" combined (CKD-EPIcysc,cr) and the two additional combined equations demonstrated sufficient accuracy with p30 >= 80% but underestimated GFR by 8 to 14%

A study by Björk showed that combining creatinine and cystatin C improved GFR estimations in adult non-ICU patients with results of p30 >= 90%[46]

Suggesting that neither the pure creatinine nor the pure cystatin C-based equations for estimating GFR are particularly useful in critically ill patients in ICU with longer treatment times. The combined formulas strengthen the accuracy though they still tend to underestimate GFR.

5.4 STUDY IV

Results

Table 9. Baseline characteristics of all recruited AKI patients.

Baseline characteristics of all recruited AKI patients (274)	Median values and IQR unless otherwise stated
Median age (years) (IQR)	64 (53–72)
Sex (female), *N* (%)	114 (41.6)
Length of stay (days) (IQR)	6 (3–12)
SAPS-2 score (IQR)	48.5 (38–64)
Invasive ventilation, *N* (%)	109 (40)
Dialysis on ICU, *N* (%)	66 (24)
Maximum urea (mmol/l) (IQR)	15.7 (9–25.2)
Baseline creatinine	
N measured (%)	156 (56.9)
Measured (μmol/l) (IQR)	64 (50.5–76)
Estimated[a] (μmol/l) (IQR)	88 (71–97)
N = 146	
Admission creatinine (μmol/l) (IQR)	135 (104–213)
Maximum creatinine (μmol/l) (IQR)	169.5 (122–263)
Last ICU creatinine (μmol/l) (IQR)	107 (72–149.5)
Admission cystatin C (mg/l) (IQR)	1.58 (1.1–2.35)
Maximum cystatin C (mg/l) (IQR)	2.14 (1.44–3.04)
Last ICU cystatin C (mg/l) (IQR)	1.65 (1.23–2.21)
Discharge creatinine/cystatin C ratio (IQR)	7.1 (5.2–9.2)
COPD	53 (14.5)
Diabetes mellitus I and II	54 (19.7)
Cardiovascular disease	90 (33.0)
Hypertension	120 (44.0)
Liver failure	99 (36.0)
Haematological malignancy	19 (6.9)
Other malignancies	84 (30.6)
Heart failure	38 (13.8)

MDRD = modified diet in renal disease formula. [a]Creatinine was estimated using the Modified Diet in Renal Disease (MDRD) formula using an expecting GFR of 75 ml/kg/min/1.73 m^2.

ICU mortality among AKI patients was 11.1% Follow-up occurred at 101.5 days (median, IQR 89.5 - 126). The median age of recruited AKI patients was 64 years (41.6% female). Median SAPS-2 was 48.5.

Measured baseline creatinine was 64 μmol/l and estimated creatinine baseline creatinine was 88μmol/l. Cystatin C was available in 211 patients with a median of 1.33mg/l.

Using cystatin C-based estimate 63.7% of patients fulfilled the criteria for KDIGO CKD stage 3 or higher. Of the creatinine-based estimators; LM-REV 30.8%, MDRD 25.8%, and CKD-EPI(creatinine) 25.8% fulfilled the CKD criteria. The composite CKD-EPI(creatinine, cystatin C) identified 42.2% of patients having CKD.

47 (18.7%) of 252 patients, who had follow-up creatinine measured between two and seven months, fulfilled the AKD criteria. Of 201 patients with both markers available, 38 (18.9%) fulfilled the CKD criteria (table 10).

Table 10. Categorisation of the cohort according to the AKD group. 201 patients where creatinine and cystatin C were both measured at follow-up (between 2 to 7 months).

Acute kidney disease grade	*N*	%
0	82	40.8
0 B-C	81	40.3
1	26	12.9
2	8	3.98
3	4	1.99
AKD grade 1–3	38	18.9

CKD diagnosis based on cystatin C-based eGFR was associated with increasing age, diabetes, and elevated discharge cystatin C. The risk of developing CKD according to creatinine-based eGFR was associated with increasing age and female sex in the logistic regression models.

One-year mortality for all AKI patients was 28.2% (18.7% in non-AKI patients). Two-year mortality for patients classified as AKD was 16.7% and 11.2% for patients without AKD (not significant). The difference in mortality between patients classified as having CKD according to creatinine or cystatin C based CKD was not significant.

Discussion

This study showed that renal dysfunction was common among patients with AKI in the ICU at follow-up at three months after discharge. I.e., CKD is more common than our national registers. Could it be underdiagnosed even in the non-AKI population? A control group could have shead light on that question. If composite eGFR equations were used it could mean that CKD incidence is even higher. In study III of this thesis, it was shown that cystatin C-based markers (admittedly in ICU context only) underestimate GFR. In study II we showed that creatinine was poorly related to the risk of death but cystatin C (at ICU discharge) on the other hand showed to be better at predicting creatinine-based eGFR. This was significant for the GFR interval 30-60ml/min/1.73m2. An inherent weakness of creatinine especially in the ICU is the coupling to sarcopenia. Cystatin C on the other hand seems to increase successively during the entire ICU admission.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Study I and III

Both studies originate from the same cohort from two ICUs in the same hospital. Recruitment was restricted to weekdays (only a few patients were included during weekends) and tampered by planned or acute treatments/examinations. Also, laboratory unavailability was a restricting factor. Selection bias may have been the most pronounced systematic error introduced into these two studies. We manually selected each patient according to in- and exclusion criteria.

The method by which GFR is measured in study III was associated with a certain amount of "eyeballing" so to speak. To interpret the elimination curves we needed to find where the curve transitions from being dominated by the fast distribution phase to the slow elimination phase. This usually happens after approximately 1-2 hours but differs depending on the individual patient's kidney function and distribution volume.

Study II

Study II was a single center retrospective observational study and as such a multicenter study would be needed to confirm the findings. All patients in the Clinisoft database during the study period were included.

However, generalizability is limited to the fact that it is a single center study. Cystatin C measurements were limited to the ICU, and the discharge measurement reflects relative clinical stability since we expect the renal recovery to be ongoing at this point. An increased cystatin C production that is independently associated with increased mortality cannot be excluded.

Study IV

This study was a single center investigation with patients from only one general ICU. The patient selection was affected by staff being unavailable and weekend or evening discharges. In the absence of baseline creatinine, it was estimated by use of the eGFR 75 approach as recommended by the ADQI group utilizing the MDRD equation. This method may introduce a misclassification of, e.g. patients with undiagnosed preexisting CKD thereby overestimating the incidence of AKI. The loss to follow-up could very well have added to the selection bias.

6.2 CONCLUSIONS

The general aim was to investigate the performance of kidney function markers in patients during critical illness. The main findings were as follows.

Study I: The coefficient of variation was greater for creatinine than for cystatin C. Both markers are within the normally acceptable range.

Study II: Levels of cystatin C after critical illness were strongly associated with 90-day and 1-year mortality both in AKI and non-AKI patients. Creatinine was poorly related to the risk of death. Creatinine had little value as a prognostic marker in the majority of patients.

Study III: Combined formulas using both creatinine and cystatin C enables the best agreement between estimated and measured GFR.

Study IV: The incidence of CKD (eGFR<60) in ICU patients three months after AKI was 25.8% when using creatinine-based eGFR and 63.7% using cystatin C-based eGFR.

Creatinine-defined CKD at follow-up was predicted by age, discharge cystatin C, discharge creatinine, and female sex.

Cystatin C-defined CKD at follow-up was predicted by age, discharge cystatin C, CRRT in ICU, and diabetes.

7 ACKNOWLEDGEMENTS

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